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Energy Metabolism During Larval Development of Green and White Abalone, *Haliotis fulgens* and *H. sorenseni*

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Abstract. An understanding of the biochemical and physiological energetics of lecithotrophic development is useful for interpreting patterns of larval development, dispersal potential, and life-history evolution. This study investigated the metabolic rates and use of biochemical reserves in two species of abalone, *Haliotis fulgens* (the green abalone) and *H. sorenseni* (the white abalone). Larvae of *H. fulgens* utilized triacylglycerol as a primary source of endogenous energy reserves for development (~50% depletion from egg to metamorphic competence). Amounts of phospholipid remained constant, and protein dropped by about 30%. After embryogenesis, larvae of *H. fulgens* had oxygen consumption rates of 81.7 ± 5.9 (SE) pmol larva^{-1} h^{-1} at 15 °C through subsequent development. The loss of biochemical reserves fully met the needs of metabolism, as measured by oxygen consumption. Larvae of *H. sorenseni* were examined during later larval development and were metabolically and biochemically similar to *H. fulgens* larvae at a comparable stage. Metabolic rates of both species were very similar to previous data for a congener, *H. rufescens*, suggesting that larval metabolism and energy utilization may be conserved among closely related species that also share similar developmental morphology and feeding modes.

Introduction

Most marine invertebrates have a distinct larval phase in their early life histories and can be divided into species whose larvae feed in the plankton (planktotrophic) and species whose larvae can develop and metamorphose without feeding (lecithotrophic) (Thorson, 1950; Strathmann, 1985). Understanding the evolution of these developmental modes within and among closely related taxa has been a major research focus in larval biology (Strathmann, 1985; Havenhand, 1995; Wray, 1995). A number of studies have used mathematical models to explore the ecological and evolutionary forces that select for planktotrophy or lecithotrophy (Vance, 1973; Christiansen and Fenchel, 1979; Caswell, 1981; Pechenik, 1987). Lecithotrophic development is believed to have repeatedly evolved from planktotrophic larval forms in echinoderms and many other phyla (*e.g.*, Strathmann, 1985; Emlet, 1990, 1995). Many changes associated with developmental mode have been reported for egg size (Emlet *et al.*, 1987), biochemical composition (Hoegh-Guldberg and Emlet, 1997; Byrne and Cerra, 2000), patterns of embryogenesis (Raff, 1987; Emlet, 1995; Martindale and Henry, 1995), and larval morphology (Olson *et al.*, 1993; Emlet, 1995).

While there are many suites of morphological and ecological characters associated with planktotrophy and lecithotrophy, less is known about the biochemistry and physiology of larval stages. A number of studies have examined the energetics of development of planktotrophic and lecithotrophic species (*e.g.*, Crisp *et al.*, 1985; Gallager *et al.*, 1986; Dawirs, 1987; Anger *et al.*, 1989; Nates and McKenney, 2000; Marsh *et al.*, 2001), and some have compared the physiological energetics of development of congeneric planktotrophic and lecithotrophic species (*e.g.*, Hoegh-Guldberg and Emlet, 1997; Moreno and Hoegh-Guldberg, 1999) as a means of exploring the evolutionary changes that are associated with the switch from feeding to nonfeeding development.

Further studies of larval energetics within closely related

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lecithotrophs will be helpful in understanding this developmental mode. The genus *Haliotis* (Gastropoda: Haliotidae), or abalone, provides an excellent model system for examining the energetics of lecithotrophic development. Large adult females produce tens of millions of eggs, providing sufficient material for biochemical and metabolic analyses of larvae throughout development. The energetics of the larvae of the red abalone (*Haliotis rufescens* Swainson) have been studied previously (*e.g.*, Jaeckle and Manahan, 1989a, b; Shilling *et al.*, 1996; Vavra and Manahan, 1999), providing a basis for comparisons of the energetics of larval development within the genus. On the basis of morphological characters, the larval development of abalone appears to be highly conserved within the genus; larvae of different species are visually very similar, and those that have been studied in detail undergo similar developmental sequences (Crofts, 1937; Ino, 1952; Seki and Kanno, 1981; Leighton, 2000). As with other archaeogastropods (Hadfield *et al.*, 1997), abalone larvae are nonfeeding. Nonfeeding larvae have been considered to depend on endogenous egg reserves to supply energy for development (Vance, 1973; Strathmann, 1985). Nonfeeding larvae can also augment energy reserves through the uptake of dissolved organic material from seawater (Jaeckle and Manahan, 1989a, b).

The two abalone species examined in this study are the green abalone, *Haliotis fulgens* Philippi, and the white abalone, *H. sorenseni* Bartsch. Both species range from central California to southern Baja California (Abbott and Haderlie, 1980) and have a history of commercial and recreational harvest. The heavy commercial harvesting of *H. sorenseni* has probably contributed to the near extinction of this species and its recent (summer 2001) listing under the Federal Endangered Species Act in the United States. Leighton (2000) has described general features of the larval development of both *H. fulgens* and *H. sorenseni*. Developmental rate is highly dependent on temperature (Leighton, 2000), and the total planktonic developmental time for both species ranges from 3 days at the upper end of their thermal tolerance to 15 days or more at colder temperatures. Here we present the developmental energetics of the larvae of *H. fulgens* and *H. sorenseni*, and compare these results to those for a third congener, *H. rufescens*, the red abalone.

Materials and Methods

Culture

Embryos and larvae were maintained in stirred cultures at 15 ± 2 °C (for *H. fulgens*) or 13 °C (for *H. sorenseni*) in UV-irradiated seawater filtered to 0.2 μm that was changed every 2 days. Larvae were reared at approximately 5 ml^{-1} in either 20- or 200-l vessels, depending on the numbers of larvae in culture. Under these conditions, by day 7 the larvae of *H. fulgens* reached the swimming and crawling stages and had branched cephalic tentacles, a character that indicates metamorphic competence in abalone larvae (Seki and Kanno, 1981). This rate of development is consistent with other studies of this species (Leighton, 2000). Biochemical samples were taken until day 8 and respiration was measured through day 9; thus our results cover the entire course of larval development. Due to limited availability of larvae of the rare white abalone *H. sorenseni*, measurements were made only on 7- to 10-day-old larvae.

Respiration

Respiration rates of embryos and larvae from two cultures of *H. fulgens*, started from gametes obtained from different parents, were measured throughout development to day 9. For larvae of *H. sorenseni*, respiration was measured daily from days 7 to 10. Larval respiration was measured with high replication (n = 10 to 16) using the end-point determination methods of Marsh and Manahan (1999). In brief, larvae were removed from cultures and suspended in fresh-filtered seawater (0.2 μm) in small respiration chambers of known volume (~500 μl, where each respiration chamber was individually calibrated). Different numbers of individuals were added to each chamber to test for concentration-dependent effects on respiration (none were observed over the range of animals used to calculate respiration rates: Fig. 1). Replicates of 10–16 chambers containing larvae were used for each set of stage-specific measurements. Larvae were incubated in the respiration chambers for 2–4 h, after which 300-μl subsamples were taken from each chamber with a temperature-equilibrated gas-tight syringe. Oxygen tension was measured in each sample with a polarographic oxygen sensor (Model 1302, Strathkelvin). Larvae in each chamber were then counted, and oxygen consumption per animal was calculated as the

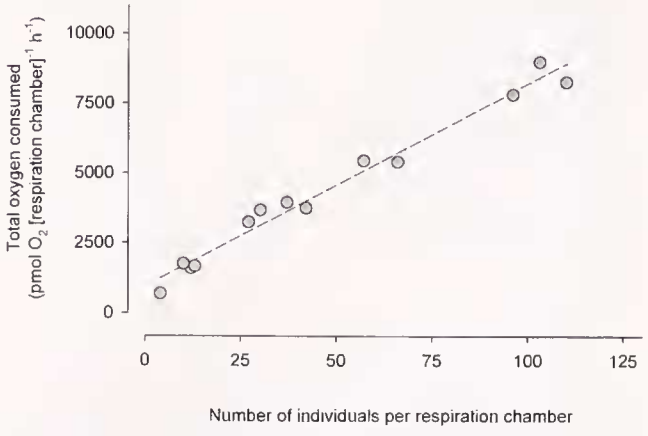

Figure 1. Oxygen consumed by 7-day-old larvae of *Haliotis fulgens* as a function of numbers of larvae used to estimate the respiration rate per individual from the slope (y = 76.3 x + 842; r^2 = 0.98; standard error of slope = 3.4). Each data point represents the oxygen consumed by larvae in a single respiration chamber (500 μl) over 4 h.

slope of the regression line of oxygen consumed per hour against number of larvae in each chamber. The error of each estimate was calculated as the standard error around the slope of the regression line.

Biochemical analysis

The conversion and interpretation of mass-to-energy equivalents in marine invertebrates has long been studied, and remains difficult (*e.g.*, Paine, 1971; Gnaiger and Bitterlich, 1984; Jaeckle and Manahan, 1989a; Moreno *et al.*, 2001). Previously in our laboratory, ash-free dry weight was used successfully as an index of total organic content in larvae of the red abalone, *H. rufescens* (Jaeckle and Manahan, 1989a; Shilling *et al.*, 1996); techniques and instrumentation were identical to those used in the present study. For both species of abalone studied here, we attempted to measure ash-free dry weight but found that the samples never reached a stable dry weight, even after 3 months of drying at 60 °C. As a result, we used direct measurements of carbohydrate, lipid, and protein to determine the general patterns of energy utilization throughout development.

Carbohydrates. Known numbers of animals (500–1000, depending on developmental stage) were aliquoted into 1.7-ml microcentrifuge tubes and centrifuged; seawater was aspirated, and the samples were frozen at −80 °C for later analysis. Total carbohydrates were quantified using the methods of Holland and Gabbott (1971). In brief, samples of eggs (day 0) and metamorphically competent larvae (day 8) were extracted with cold 5% trichloroacetic acid. The supernatant was hydrolyzed for 2 h at 95 °C in 1*M* HCl, then carbohydrates were spectrophotometrically quantified with a ferricyanate reduction reaction using glucose as a standard.

Protein. Samples of known numbers of eggs or larvae were processed as above, and protein was analyzed with the methods of Bradford (1976) as modified by Jaeckle and Manahan (1989a). Protein content was calculated by dividing the total protein in a sample by the total number of animals in that sample. The rate of protein loss during development was estimated as the slope of the regression of total protein per individual over time. Total protein loss during development was calculated as the amount available initially in the egg minus the amount present in an 8-day-old larva.

Lipids. Samples of known numbers of eggs or larvae were collected and processed as above for carbohydrate and protein. For analysis, samples were sonicated, and lipids were extracted using the methanol:chloroform:water extraction of Bligh and Dyer (1959) as modified by Holland and Gabbott (1971) and Holland and Spencer (1973), with additional modifications listed here. Lipids were extracted for 2 h in 2:2:1 (v/v/v) water:methanol:chloroform to which stearyl alcohol had been added as an internal standard. Stearyl alcohol had been previously determined not to interfere with native lipid peaks in abalone larvae (Fig. 2). Phases were separated by the addition of water and chloroform to a final ratio of 4:2:3 water:methanol:chloroform, followed by a centrifugation at 8,000 × *g*. The lower phase was isolated and dried down under nitrogen, and samples were redissolved in a known volume of chloroform. To quantify lipid classes, four replicates were taken at each sampling interval during development. For each replicate, the following procedure was repeated four times. A 1-μl sample of the redissolved lipid in chloroform was taken with a 1.5-μl gas-tight syringe (Precision Sampling Corp.) and applied to a thin-layer chromatography (TLC) "Chromarod" of 1-mm diameter (Iatron Laboratories, Inc). Chromarods were developed in a solvent system of 60:6:0.1 hexane:diethyl ether:formic acid to separate major lipid classes, dried, and immediately analyzed with an Iatroscan MK-5 flame ionization detector (FID). Calibrations were performed for each compound class, using L-α-phosphatidylcholine (phospholipid), cholesterol, tripalmitin (triacylglycerol), lauric acid myristyl ester (wax ester), and stearyl alcohol (fatty alcohol internal standard). Peaks were quantified using E-Lab (OMS Tech, Inc.) chromatography software on a personal computer. The amount of lipid per individual was calculated for each class as the mean of each of the four replicates divided by the number of individuals per sample. Total lipids were calculated as the sum of major lipid classes.

Standards for neutral lipid quantification. Methods for the quantification of specific biochemical components, as above, use an appropriate standard of known composition to quantify the contents of organisms that have unknown or mixed biochemical compositions. For lipid analysis of marine organisms known to have fatty acids with different degrees of carbon-bond saturation, the percent of unsaturation might confound interpretations of lipid content when

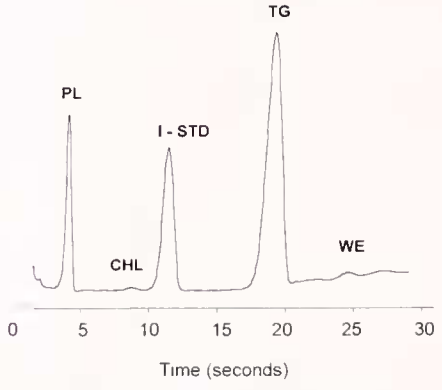

Figure 2. Chromatograph of lipids extracted from eggs of *Haliotis fulgens*. The ordinate is measured in arbitrary units (volts) of peak height. Lipid profile shows large peaks of triacylglycerol (TG) and phospholipid (PL), small peaks of cholesterol (CHL) and wax ester (WE), and the fatty alcohol internal standard stearyl alcohol (I - STD).

using standards that contain a different degree of unsaturation relative to the unknown sample. We addressed this issue of lipid standards and calibration of the FID response in this study of the role of lipids in larval energy metabolism. The peak areas of known standards were compared after chromatographic separation by TLC and quantification by FID of identical quantities of fatty acids containing different numbers of double bonds (unsaturation). Quantification by FID is dependent upon the degree of saturation of each fatty acid. Thus a different FID response per unit mass of lipid containing different numbers of double bonds would be predicted. In an analysis of lipid calibration standards conducted in our laboratory (M. Moore and D.T. Manahan, unpubl. data), 1 μg of each fatty acid was loaded onto a TLC "Chromarod" and quantified by FID for (1) palmitic acid, a fully saturated lipid with no double bonds; (2) oleic acid, with 1 double bond; (3) linoleic acid, with 2 double bonds; and (3) arachidonic acid that contains 4 double bonds. The amount of lipid was quantified by FID using palmitic acid as the 100% calibration standard for each set of samples (n = 3–6). Each sample contained the same quantity of fatty acid but had different numbers of double bonds (levels of saturation). The following values were measured for each fatty acid (values are mean ± SD): palmitic acid, no double bonds = 1.0 μg ± 0.13; oleic acid, 1 double bond = 0.9 μg ± 0.12; linoleic acid, 2 double bonds = 0.8 μg ± 0.08; arachidonic acid, 4 double bonds = 0.6 μg ± 0.12. These data show a trend of decreasing FID response with increasing number of double bonds. Within analytical error, however, there was no significant difference in the FID response for fatty acids with 0, 1, or 2 double bonds (VR = 2.99^{ns} for 2, 12 df). This full analysis was repeated with an additional set of standards, giving a similar result that showed no significant effect of 0, 1, or 2 double bonds on the quantification of fatty acids (VR = 2.89^{ns} for 2, 9 df). For both sets of replicate samples, there was a significant difference for an ANOVA that included arachidonic acid (4 double bonds) in the analysis of FID response to 0, 1, 2, and 4 double bonds. The calibration study was also expanded beyond an analysis of monomers: a comparison of standards based on tripalmitin and triolein also showed no significance difference at 1.00 μg ± 0.04 and 0.97 μg ± 0.04, respectively.

Larvae of *H. fulgens* have 42% of their fatty acids in a fully saturated form, with palmitic acid being the dominant fatty acid at 30% (Nelson *et al.*, 2002). An additional 42% of the fatty acids are monounsaturated—a chemical difference that is not statistically distinguishable from saturated fatty acids by FID in our analysis (as above). Only 13% of the total fatty acids present in larvae of *H. fulgens* tissues are polyunsaturated, and this percent is not likely to cause significant error if tripalmitin is used as a standard for neutral lipid analysis (as in our study). Given the statistical analysis above of the comparison of different fatty acids as standards, we conclude that the use of tripalmitin as a standard for larvae of *H. fulgens* will result in an insignificant error for the quantification of total neutral lipid content.

Results

The respiration rates of larvae of *Haliotis fulgens* were similar for the two cultures spawned from different sets of parents. Larvae in Culture 1 reached the veliger stage by day 2, and their respiration rates through subsequent larval development remained fairly constant, with a mean rate of 83.4 ± 2.6 (SE) pmol O_2 $larva^{-1}$ h^{-1} (Fig. 3). For Culture 2, the mean respiration rate was 80.0 ± 5.3 pmol O_2 $larva^{-1}$ h^{-1} (Fig. 3). Within a specific culture, the respiration rates of pre-veliger stages (< 2-day-old) were lower than in later veliger stages. For *H. sorenseni*, the mean respiration rate of larvae was 58.9 ± 4.7 pmol O_2 $larva^{-1}$ h^{-1} (Fig. 3).

The amount of protein in the eggs from the two spawns of *H. fulgens* was similar (Fig. 4, Day 0). The rate of decrease in total protein during development was not significantly different between the two cultures: by analysis of variance of compared regressions, the variance ratios between the two slopes and the two y-axis intercepts were 0.25 and 0.73, respectively (not significant for 1, 13 df). The data for both cultures were combined into a single regression to calculate a rate of protein loss of 13 ng $individual^{-1}$ day^{-1}, resulting in a total loss of about 30% from the initial content in the egg to 8-day-old larvae (Fig. 4).

Lipid-class analysis of *H. fulgens* showed that triacylglycerol was the dominant class, followed by phospholipid (Fig. 2). Cholesterol and wax ester contents were very low (below quantifiable detection limit: Fig. 2) relative to tri-

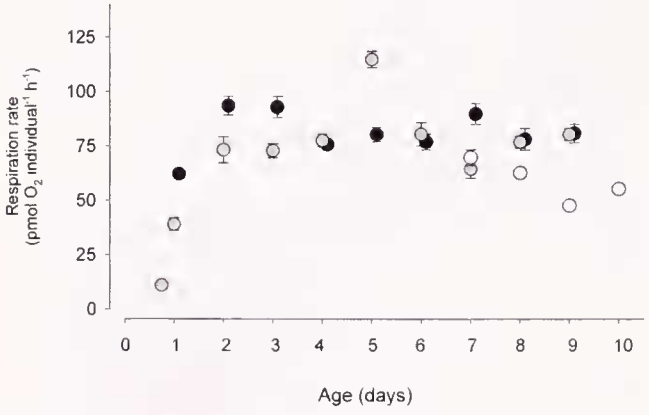

Figure 3. Rates of oxygen consumption over development of larvae from Culture 1 (black symbols) and Culture 2 (grey symbols) of *Haliotis fulgens*, and from day 7 to day 10 of development for larvae of *H. sorenseni* (white symbols). Respiration rates were measured at the rearing temperatures of both species: 15°C for *H. fulgens* and 13°C for *H. sorenseni*. Error bars are the standard error of the slope used to estimate respiration rates of individual larvae (see Fig. 1).

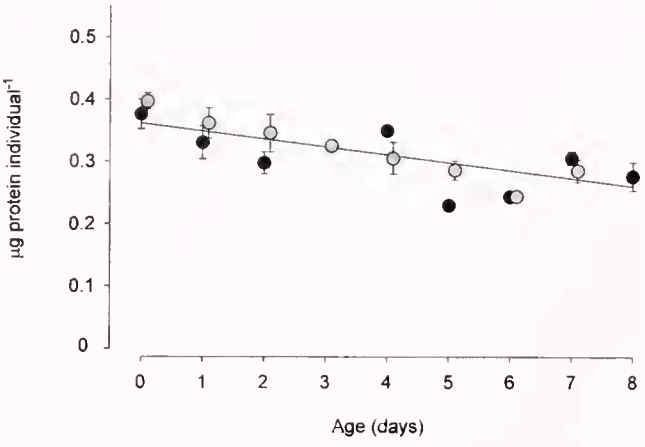

Figure 4. Total protein content during development of *Haliotis fulgens*. Data are from two cultures: Culture 1 (black symbols) and Culture 2 (grey symbols). Each data point represents the mean of 4 independent samples (day 0 = eggs). The rate of protein decline was not different between the two cultures (see text), and all data were combined to calculate the rate of protein loss over development: μg protein individual^{-1} = $-0.013x + 0.361$, where x represents age in days (error bars are ± 1 SE).

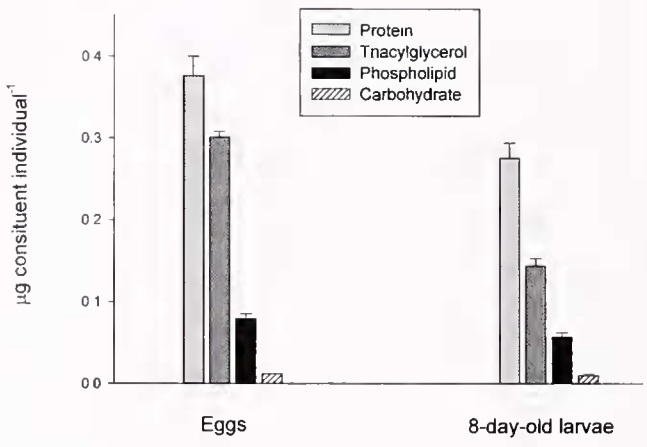

Figure 6. Protein, triacylglycerol, phospholipid, and carbohydrate content of *Haliotis fulgens* measured at day 0 (eggs) and day 8 (late-stage larvae, Culture 1). Error bars are the standard error.

acylglycerol and phospholipid, and are not included in presentation of the developmental changes in lipid content (Fig. 5). In larvae from both cultures of *H. fulgens*, phospholipid content remained constant throughout development, while triacylglycerol decreased by about 50% (Fig. 5). Larvae of *H. sorenseni* had 0.40 ± 0.02 μg protein, 0.36 ± 0.03 μg triacylglycerol, and 0.13 ± 0.01 μg phospholipid per larva (7-day-old).

Carbohydrate content was low. *H. fulgens* contained 1.2 ± 0.05 ng carbohydrate egg^{-1} and 1.0 ± 0.23 ng larva^{-1}. These amounts corresponded to only 1.6% and 2.1% of the total protein and lipid content of eggs and larvae, respectively (Fig. 6).

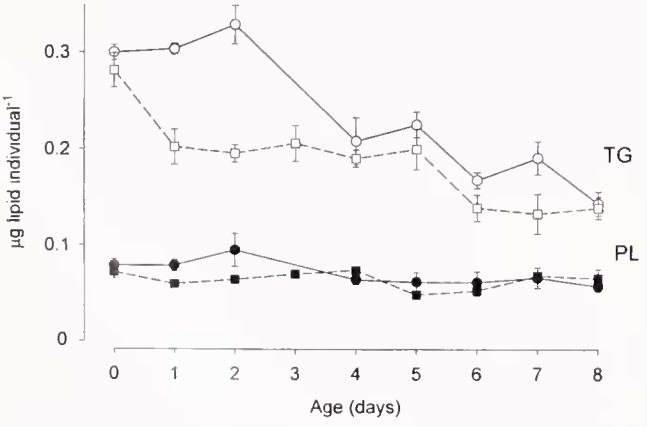

Figure 5. Lipid content of *Haliotis fulgens* larvae over development for Culture 1 (circles) and Culture 2 (squares). White symbols represent triacylglycerol; black symbols represent phospholipid (error bars are ± 1 SE).

Discussion

Energy metabolism and rates of utilization of endogenous reserves in marine invertebrates with planktotrophic larval forms have received considerable attention (*e.g.*, Crisp, 1974; Holland, 1978; Gallager *et al.*, 1986; Anger *et al.*, 1989; Shilling and Manahan, 1994; Marsh *et al.*, 2001). However, fewer species with lecithotrophic modes of development have been studied in similar detail (*e.g.*, echinoderms: Hoegh-Guldberg and Emlet, 1997; crustaceans: Anger, 1996; bryozoans: Wendt, 2000). This has made it difficult to make comparisons and generalizations about the energetics of nonfeeding development, or to understand the role of larval energetics in the evolution of the life-history strategies of marine invertebrates. The genus *Haliotis* offers several advantages as a taxon for studying the comparative energetics of lecithotrophic development among closely related species. All larval forms studied to date within this genus are lecithotrophic, and the findings presented here show that patterns of energy utilization are similar among several species in this genus. Specifically, the biochemical compositions of larvae of the green (*H. fulgens*; this study), white (*H. sorenseni*; this study), and red (*H. rufescens*; Jaeckle and Manahan, 1989a) abalone are similar, with protein and lipid being the dominant macromolecules. Likewise, the metabolic rates of these three species of abalone larvae are similar, even given the 2 °C difference in temperature between the green and white abalone larvae (Fig. 3 this study; *cf.* red abalone larvae at 17 °C = 84 pmol O_2 larva^{-1} h^{-1}: Jaeckle and Manahan, 1989a).

Of the endogenous components used to fuel development in marine invertebrate larvae, lipids are generally considered to be more important than protein and carbohydrates (Holland, 1978). Our results are consistent with

this conclusion for carbohydrates. In eggs and metamorphically competent larvae of *H. fulgens* (Fig. 6), carbohydrates are not a major energy source, making up about 2% of the total content of lipid and protein. In contrast, and in agreement with other biochemical analyses of lecithotrophic larval development, our results also show that lipid is a major source of energy. During larval development from egg to a stage competent to metamorphose (Fig. 5), triacylglycerol decreased while phospholipids remained constant. This pattern is consistent with ontogenetic changes in lipid composition during nonfeeding development of some other marine organisms, in which triacylglycerol decreased while the polar lipid fraction changes little over development (*e.g.*, Takii *et al.*, 1997; Whertmann and Graeve, 1998; Nates and McKenney, 2000). A major portion of the metabolic costs of abalone larval development can be explained by catabolism of triacylglycerol (see below). From Figure 5, taking the amount of triacylglycerol in the egg (Culture 1: 0.30 μg) and subtracting the triacylglycerol content of 8-day-old larvae (0.14 μg), a total of 0.16 μg of lipid was depleted over development. When converted to energy equivalents (39.5 kJ g^{-1}; Gnaiger, 1983), lipid catabolism could provide 6.3 mJ of energy per larva. A similar set of calculations resulted in 5.5 mJ of energy for a larva from Culture 2 (energy equivalents from Gnaiger, 1983). The energetic cost of larval development from Figure 3, based on cumulative oxygen consumption to day 8 (for comparison with lipid depletion to day 8), was 15.6 μmol O_2 $larva^{-1}$ (Culture 1) and 14.4 μmol O_2 $larva^{-1}$ (Culture 2). Based on the oxyenthalpic equivalent of lipid catabolism [441 kJ (mol O_2^{-1})] (Gnaiger, 1983), these oxygen consumption values are equivalent to 6.9 mJ and 6.3 mJ per larva for Culture 1 and Culture 2, respectively. Hence, the energy made available from the depletion of lipid could account for 91% and 87% of metabolism for larvae in Cultures 1 and 2. These calculations support the conclusion that measured depletion of lipid reserves could fuel most of the larval development of *H. fulgens*. This is consistent with the larval energetics of other marine molluscs, in which lipids are a major source of endogenous energy (Millar and Scott, 1967; Holland and Spencer, 1973; His and Maurer, 1988).

In addition to lipid, total protein content also decreased in larvae of *H. fulgens* over development from egg to competent larva (Fig. 4: day 8). Subtracting the protein content of 8-day-old larvae (0.28 μg) from the protein content of the egg (0.38 μg) indicated that larvae in Culture 1 lost a total of 0.1 μg protein $individual^{-1}$. Larvae in Culture 2 also utilized 0.1 μg protein; in both cultures, when this protein loss was converted to energy equivalents of complete oxidation (24.0 kJ g^{-1}), it was equivalent to 2.4 mJ $individual^{-1}$ over 8 days. Clearly, the total energy available to a larva from the depletion of lipid and protein combined (Culture 1 = 8.7 mJ; Culture 2 = 7.9 mJ) could theoretically exceed the metabolic demand as measured by oxygen consumption for larvae of *H. fulgens*. In addition, biochemical fractions not measured as total protein, lipid, and carbohydrate (Holland and Gabbott, 1971; Gnaiger and Bitterlich, 1984) could also contribute to metabolism. The *actual* contribution, however, of any metabolic substrate to metabolism depends on the extent to which that substrate is fully oxidized to release energy. Also, the extent to which the proportional loss of different substrates can be assigned the correct oxyenthalpic equivalent for energy-yield calculations depends on the extent of full oxidation [*e.g.*, protein = 527 kJ (mol O_2^{-1}) *cf.* lipid = 441 kJ (mol O_2^{-1})]. Assumptions about the identity of metabolic substrates and their degree of oxidation are commonly used to calculate energy budgets for marine invertebrate larvae. If, however, not all materials depleted during development are fully oxidized, these assumptions will not be entirely accurate. To fully describe the energetics of larval development, future studies should not only consider oxygen consumption and depletion of specific macromolecules, but also include measurements of the actual rates of oxidation of these biochemical constituents. To our knowledge, this suite of measurements has never been simultaneously taken in larvae of any marine invertebrate species.

For larvae of *H. fulgens*, the energy available from the presumed 100% catabolism of energy reserves was more than adequate to support metabolic demand. This is in contrast to previous studies with other species of abalone larvae (*H. rufescens:* Jaeckle and Manahan, 1989a), in which metabolic demand exceeded energy available from depletion of protein and lipid over development. The chemistry of the seawater used to culture larvae is likely to be important in lecithotrophic larval energetics. The larvae of many soft-bodied marine invertebrates can take dissolved organic material (DOM) up directly from seawater (Manahan, 1990). Both in laboratory and field situations, low concentrations of DOM may force larvae to catabolize endogenous reserves to fuel development, while larvae in DOM-rich seawater may be able to utilize those exogenous sources of energy, thereby sparing endogenous reserves (Jaeckle and Manahan, 1992). Differences in patterns of utilization of endogenous energy reserves among different studies of larvae—for example, the lack of a decrease in lipid during larval development of *H. rufescens* (Jaeckle and Manahan, 1989a)—are most likely attributable to environmental issues, such as the organic "quality" of the seawater used to rear the larvae. Variations in the seawater "quality" used to culture larvae and differences in maternal inputs to egg "quality" (Buchal *et al.*, 1998) may overwhelm small species-specific differences in biochemical composition and larval energy metabolism among closely related organisms with similar life histories.

Acknowledgments

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